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Study of the Protein Patterns under the Effect of Spraying with Moringa Plant Extract and Potassium Silicate for Banana Obtained from Tissue Culture *Musa* spp.

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The field experiment was conducted in one of the greenhouses affiliated with the College of Agriculture and Marshes - University of Thi-Qar during the growing season 2022-2023 on banana seedlings, the Grand Nine variety, to know the effect of Moringa extract and potassium silicate on the protein patterns of seedlings. The study included two factors. The first factor was spraying with an extract. Moringa leaves in four concentrations (0, 5, 10, 15) g L-1, the second factor: spraying with potassium silicate in four concentrations (0, 1, 2, 3) ml L-1, the first spraying appointment was at an interval of ten days between one spray and another, by 6 sprays, the experiment was applied according to the complete random design (C.R.D) with three replications. The results for the protein patterns were as follows. All treatments participated in the presence of two protein bundles that shared the same molecular weight as the first protein bundle, which amounted to 143.724 kDa. It also shared the same molecular weight as the second protein bundle, which recorded a molecular weight of 93.595 kDa. It gave four protein bundles in the comparison treatment and five protein bundles. Only for all treatments of Moringa extract and all potassium silicate treatments with molecular weights ranging between 7.619-15.465 kDa, and when the interaction between Moringa extract at a concentration of 5g L-1 with all concentrations of potassium silicate caused an increase in the number of protein bundles to six bundles whose molecular weights ranged between 7.857-8.862 kDa, as for the interaction between Moringa extract at two concentrations of 10 and 15 g L-1 with all potassium silicate concentrations (except for concentration 3 ml L-1), it caused an increase in the number of protein bundles to seven, with molecular weights ranging between 7.698-8.254 kDa. While the interaction between Moringa extract 15 g L-1 and potassium silicate at a concentration of 3 ml L-1 caused an increase in the number of protein bundles to eight bundles with different molecular weights, the molecular weight of the eighth bundle was 7.619 kDa.

Keywords: Musa spp., spraying, greenhouses, moringa extract, banana seedlings, complete random design (CRD), potassium silicate.

INTRODUCTION

Bananas (Musa spp.) belong to the Musaceae family and occupy an advanced world trade position. It is cultivated throughout several continents. It is a tropical fruit crop (Agha and Dawood 1991). Banana fruits have a high nutritional importance. It is one of the fruits rich in carbohydrates, which constitute about 30% of the fresh weight of the fruits. It also contains mineral elements and vitamins. It was found that every 100 grams of fruit pulp gives 100 calories. It is also an excellent and affordable food supply. In addition, its components have pesticides, antioxidants, wine, alcohol, biogas, and fodder for livestock, and it is used in many industries (Mohapatra *et al.*, 2021). Bananas are fast-growing plants, so they require the preparation of nutrients to improve vegetative and fruitful growth. Potassium is one of the main elements that the plant needs on an ongoing basis, so it needs to be added with the two elements phosphorus and nitrogen (Turner and Lahav, 1983). In addition to the importance of potassium in regulating the osmotic potential and controlling the opening and closing of stomata, it has important roles in plant growth and development (Cresser and Parsons, 1979). Synthetic agrochemicals can be reduced as much as possible, and environmentally friendly natural materials act as biostimulants to intentions. We must point out the use of plant extracts such as moringa and algae, agricultural and industrial organic waste and nano-silicone (Matthews *et al.*, 2022).
One of the most famous natural nutritional supplements for sustainable agriculture is the moringa leaf extract through its contribution to providing the plant with nutrients and

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regulators that improve plant growth, as it contains the primary nutrients, vitamins, proteins, amino acids, phenols, flavinoids and carotenoids, in addition to containing cytokinins such as zyatin therefore, it contributes to supplying the plant with the nutrients it needs and natural growth regulators, and then increasing production in quantity and quality (Sardar *et al.*, 2021). The presence of the major nutrients that the plant needs, along with potassium silicate, which is the main source of silicon, plays important roles in plant growth and development, as well as providing quantities of potassium (Sardans and Penuelas, 2021).

Many types of genetic indicators, such as protein, enzyme, and cellular indicators, are used to describe the genetic makeup and the factors affecting it, in addition to DNA indicators, when using protein indicators. The term "genetic fingerprint" refers to the distribution of gel bands obtained from the analysis of the protein content of the studied individuals (Khairallah, 2009). A trait used to infer the presence of a particular locus (lux) on a chromosome or gene is referred to as a genetic marker, and knowledge of this site contributes to the study of the inheritance of a specific trait or gene, as genes that are very close to the marker are inherited (Mistrello *et al.*, 2008).

Furthermore, investigating the protein patterns under the effect of spraying with moringa plant extract and potassium silicate for banana obtained from tissue culture Musa spp. offers valuable insights into the molecular mechanisms underlying the response of banana plants to these treatments. Protein patterns serve as molecular signatures that can reveal changes in gene expression and metabolic pathways in response to external stimuli. By analyzing protein patterns, researchers can identify key proteins involved in plant growth, stress response, and fruit development, shedding light on the physiological and biochemical processes influenced by moringa plant extract and potassium silicate application in banana cultivation (Ramarao *et al.*, 2022; Shi *et al.*, 2019).

The integration of natural supplements like moringa leaf extract and potassium silicate alongside genetic studies utilizing protein indicators and genetic markers contributes significantly to advancing sustainable banana cultivation practices and understanding genetic traits affecting banana plants' growth and development. This holistic approach not only enhances agricultural productivity but also promotes environmental sustainability and food security (Zhu *et al.*, 2021; Zakaria *et al.*, 2024).

MATERIALS AND METHODS

In one of the greenhouses connected to the College of Agriculture and Marshes - Dhi Qar University in Nasiriyah, a field experiment was carried out during the growing season 2022-2023 on banana seedlings to know the effect of Moringa extract and potassium silicate on its prototype. The greenhouse was prepared. After preparing the agricultural medium consisting of sand and peat moss at a ratio of 1:3, the seedlings were planted inside the pots, and weeding, control and irrigation operations were carried out whenever needed. The experiment included two agents: 1- Spraying with Moringa leaf extract in four concentrations (0, 5, 10, and 15) g. L-1 and its symbol (a0, a1, a2, a3) 2- Spraying with potassium silicate compound in four concentrations (0, 1, 2, 3) ml. L-1 and its symbol (b0, b1, b2, b3). Spraying was done 6 times at intervals of 10 days. The experiment was created using a Complete Randomised Design, and the averages were compared using L.SD at 5%. The data were statistically analyzed using the statistical programme Genstat 2016.

The Protein Pattern of Leaves was Studied: The materials needed to be lyophilized using the Freeze Dryer Lyophilization Technique were put in plastic containers. They put in an Edwards model (Prianiso) lyophilization equipment at a temperature of (-26 °C). for a period of time to remove most of the moisture. Then, these samples were utilized in the electrophoresis of proteins on a polyacrylamide gel in the presence of SDS Sodium Dodecyl Sulphate, as described in (Leammli, 1970).

Protein was extracted from the lyophilized leaves by combining 1 g of lyophilized leaves Using a ceramic mortar and pestle at 4 degrees Celsius with 3 cc of Tris-HCl-buffer (0.1M, pH 7.5) containing (PMSF) Phenyl Methane Sulfonyl Fluoride. The centrifugation process was performed at (4 °C) and (18000) cycles per minute for 30 minutes, after which (40 l) of the filtrate was transferred to a polyacrylamide gel using a transfer device. According to the method (David & Nilsen 2000), in the presence of SDS teratogens. Protein was extracted from the dried leaves by combining 1 g of the leaves At 4°C-temperature and 3 cc of Tris-HCl-buffer (0.1M, pH 7.5) containing (PMSF) Phenyl Methane Sulfonyl Fluoride in a ceramic mortar. The centrifugation procedure was performed at (4°C) and (18000) revolutions per minute for 30 minutes, after which (40 microliters) of the filtrate was transferred to a Polyacrylamide gel relay.

***Electrophoresis preparations*:** Using the Slab-Electrophoresis method and SDS denaturants, protein electrophoresis was carried out on a polyacrylamide gel (David & Nilsen 2000) method. The samples were made by combining the sample's buffer solution with the precipitated protein from the sedimentation process, putting the solution in a boiling water bath for five minutes, and letting it cool at room temperature before transferring the sample. Gel separation buffer, 0.3 ml of SDS solution, 150 L of ammonium persulfate solution, and 15 L of tamd were combined with 14.55 ml of distilled water to create the separation gel. The mixture was then allowed to harden for 1.5 hours. Mix 2.6 ml of acrylamide solution with 2.6 ml of gel-stacking buffer solution in 12.2 ml of distilled water and 0.2 ml of SDS solution. Add 50 l of ammonium persulfate solution and 10 l of tamd, and allow the gel to harden. One and a half hours to prepare for electrophoresis.

***Operation of the device*:** The components of the separating gel were mixed and then carefully injected with a medical syringe between two glass plates in the chamber of the electrophoresis device. Type (Cleaver Scientific), and leave the gel for a while to complete solidification, then add the stacking gel, then put the comb designated to form pits in the gel to inject the model and leave the gel to complete solidification, then carefully lift the comb to prevent deformation in the formed pits, then inject the samples with a micro syringe Measure 50 microliters and after completing the process, put the chamber in the electrophoresis device and add a buffer solution to it, then close the device and connect to the power supply, adjust the power supply to 2.5 milliamps (70 volts) in the model stacking stage and 5 milliamps (100 volts) in the Separation phase. The transfer process lasted 3-4 hours.

***Gel removal*:** Using a small amount of water, carefully remove the gel from the two glass plates, add the dyeing solution, allow it to sit for 24 hours, then remove the gel from the dyeing basin and wash it with the dye removal solution until bands appear. It was captured using Gel documentation. The Promega company's Broad Range The molecular weights of the proteins were determined using protein molecular weight markers and plotted using the PhotoCapt Mw (17version) computer program.

***The treatments were numbered with numbers*:** 1- (marker) 2- (comparison) 3- (potassium silicate 1 ml L-1) 4- (potassium silicate 2 ml L-1) 5- (potassium silicate 3 ml L-1) 6- (Moringa 5 g L-1) 7- (Moringa 10 g L-1) 8- (Moringa 15 g L-1) 9- (Moringa 5 g L-1 + potassium silicate 1 ml L-1) 10- (Moringa 5 g L-1 + potassium silicate 2 ml L-1) 11- (Moringa 5 g L-1) + potassium silicate 3 ml L-1) 12- (Moringa 10 g L-1 + potassium silicate 1 ml L-1) 13- (Moringa 10 g L-1 + potassium silicate 2 ml L-1) 14- (Moringa 10 g L-1 + potassium silicate 3 ml L-1) 15- (Moringa 15g L-1 + potassium silicate 1 ml L-1) 16- (Moringa 15 g L-1 + potassium silicate 2 ml L-1) 17- (Moringa 10 g L-1 + potassium silicate 3 ml L-1).

RESULTS AND DISCUSSION

The findings of banana leaves' protein pattern (Fig. 1-17) indicate differences between all treatments or study conditions, as the specifications of the protein bundles depend on the treatment; each treatment varies in size, area, and height. As we can see from Fig. 8 that all treatments participated in the presence of two protein bundles that shared the same molecular weight of the first protein bundle, which amounted to 143.724 kDa, and also shared the same molecular weight of the second protein bundle, which recorded a molecular weight of 93.595 kDa, and this indicates that all banana seedlings used in the study are genetically identical.

The quantity, placement, and characteristics of the protein bundles on the polyacrylamide gel varied between the experimental treatments. Depending on the type of treatment and its concentration, there were between (4 and 8) protein bundles. There were four protein bundles in the comparison treatment and only five. For all treatments of Moringa extract and all treatments of potassium silicate with molecular weights ranging between 7.619-15.465 kDa, meaning that treatment with Moringa extract alone and treatment with potassium silicate alone caused an increase in the number of protein bundles, one bundle compared to the comparison treatment, and the reason may be due to an increase in protein concentration in these treatments.

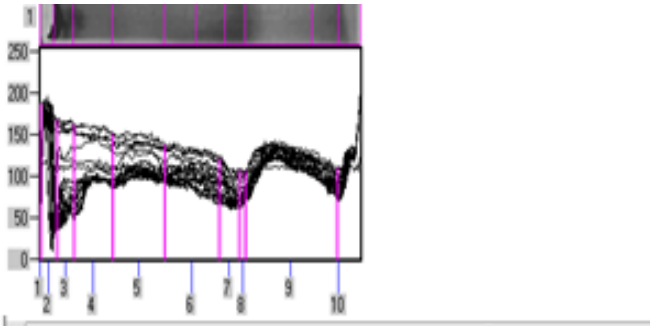
When the Moringa extract at a concentration of 5g L-1 interacted with all the concentrations of potassium silicate, it caused an increase in protein bundles to six, whose molecular weights ranged between 7.857-8.862 kDa.

As for the interaction between Moringa extract at two concentrations of 10 and 15 g L-1 with all potassium silicate concentrations (except for concentration 3 ml L-1), it caused an increase in the number of protein bands to seven, with molecular weights ranging between 7.698-8.254 kDa. The interaction between Moringa extract 15 g L-1 and potassium silicate at a concentration of 3 ml L-1 increased the number of protein bundles to eight with different molecular weights. The molecular weight of the eighth bundle was 7.619 kDa. It seems that these treatments have affected the process of gene expression and caused an increase in protein bundles; since their impact could be seen in how protein bundle positions and molecular weights changed, It suggests that they have stimulated the generation of new proteins and the activation of the gene expression process, which may play a significant role in the growth and development of banana seedlings.

These findings suggest that the usage of potassium silicate and moringa extract may accelerate the production of natural proteins and that the mechanisms of genetic transcription and translation may change, resulting in the formation of new proteins via the mechanism of gene expression, according to the requirements of the plant and its reaction to the type of treatment, so promoting the plant's development and growth (Sinha and Roy, 2002). The study's coefficients may also contribute to the activation of a specific type of genes that form RNA needed to build proteins, and this leads to an increase in the negative charge of the water potential, which reduces turgor pressure and the wall's tensile resistance. As a result, water and nutrients can seep into the cell, which leads to an increase in cell size and, thus, improved plant growth and development (Elshibi & Korpelainen, 2009).

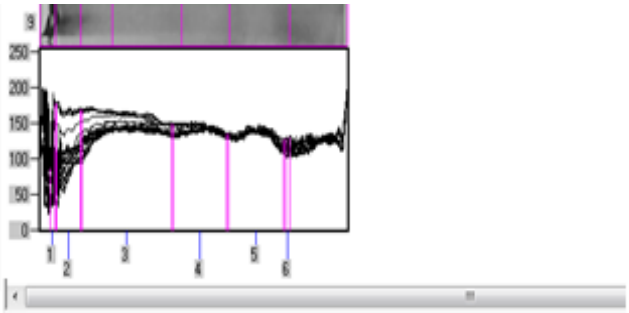
The increase in the number of protein bundles with specific treatments, especially those involving combinations of Moringa extract and potassium silicate, suggests these treatments enhance protein synthesis beyond the baseline levels observed in the control group. This enhancement in protein synthesis indicates an upregulation of gene

expression, leading to the production of new proteins necessary for the growth and development of banana seedlings. The findings suggest that both Moringa extract and potassium silicate act as biostimulants, triggering specific genetic pathways that promote the synthesis of proteins, which in turn may contribute to improved plant growth and resilience. Moreover, the study implies that the mechanisms underlying these responses involve changes in genetic transcription and translation processes, possibly through the activation of genes responsible for the synthesis of RNA and proteins. This process not only supports the production of structural proteins but may also influence the plant's metabolic pathways, enhancing its ability to uptake and utilize nutrients, thereby facilitating better growth and development.



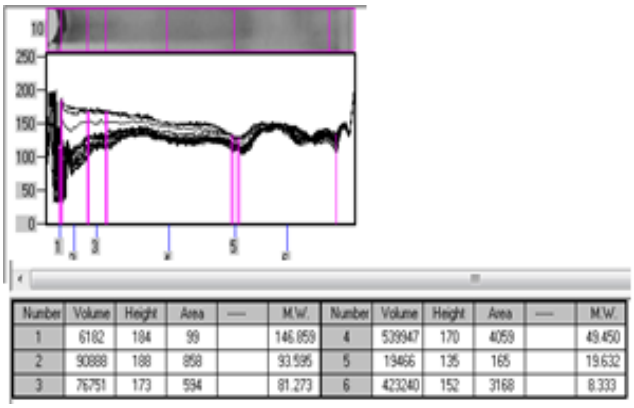
Number	Volume	Height	Area	—	M.W.	Number	Volume	Height	Area	—	M.W.
1	5663	188	32		225.000	6	174069	140	1683		35.000
2	64505	196	462		150.000	7	50751	124	594		25.000
3	43441	168	495		100.000	8	13431	109	165		15.000
4	125071	163	1188		75.000	9	313214	143	2838		10.000
5	177813	154	1617		50.000	10	2822	112	33		8.016

Figure 1. Protein bands on the polyacrylamide marker gel.



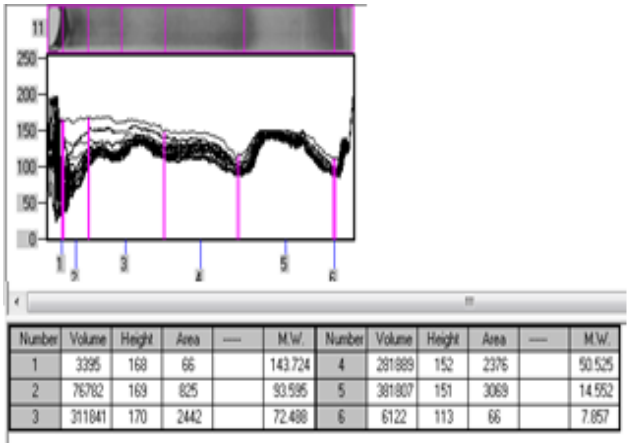
Number	Volume	Height	Area	—	M.W.	Number	Volume	Height	Area	—	M.W.
1	14579	192	198		143.724	4	244391	152	1749		38.496
2	90195	180	825		93.595	5	240574	149	1815		18.540
3	420526	173	2937		73.493	6	18972	133	165		8.862

Figure 2. A polyacrylamide gel's protein bands' measurements for interaction of Moringa extract 5 g L^{-1} with potassium silicate 1 ml L^{-1}.



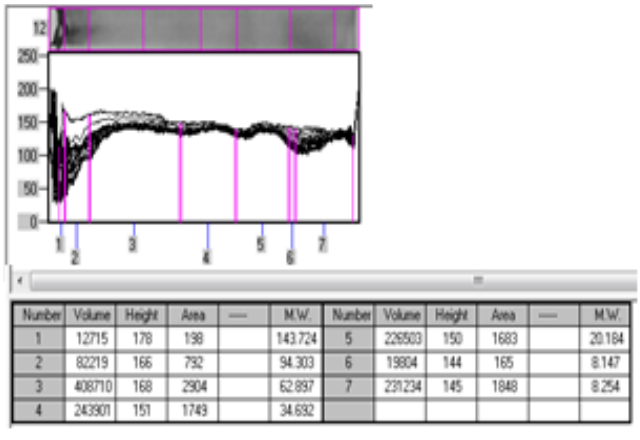
Number	Volume	Height	Area	—	M.W.	Number	Volume	Height	Area	—	M.W.
1	6182	184	99		146.859	4	539947	170	4059		49.450
2	90888	188	858		93.595	5	19466	135	165		19.632
3	76751	173	594		81.273	6	423240	152	3168		8.333

Figure 3. A polyacrylamide gel's protein bands' measurements for interaction of Moringa extract 5 g L^{-1} with potassium silicate 2 ml L^{-1}.



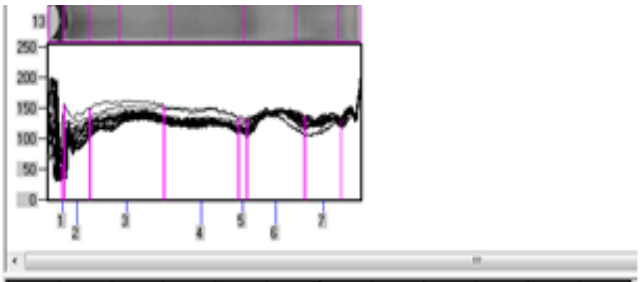
Number	Volume	Height	Area	—	M.W.	Number	Volume	Height	Area	—	M.W.
1	3395	168	66		143.724	4	281889	152	2376		50.525
2	76782	169	825		93.595	5	381807	151	3069		14.552
3	311841	170	2442		72.488	6	6122	113	66		7.857

Figure 4. A polyacrylamide gel's protein bands' measurements for interaction of Moringa extract 5 g L^{-1} with potassium silicate 3 ml L^{-1}.



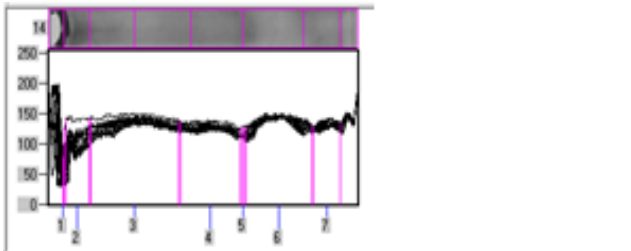
Number	Volume	Height	Area	—	M.W.	Number	Volume	Height	Area	—	M.W.
1	12715	178	198		143.724	5	226503	150	1683		20.184
2	82219	166	792		94.303	6	19804	144	165		8.147
3	408710	168	2904		62.897	7	231234	145	1848		8.254
4	243901	151	1749		34.692						

Figure 5. A polyacrylamide gel's protein bands' measurements for interaction of Moringa extract 10 g L^{-1} with potassium silicate 1 ml L^{-1}.



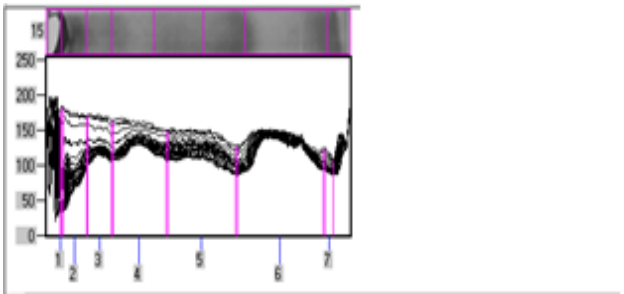
Number	Volume	Height	Area	----	M.W.	Number	Volume	Height	Area	----	M.W.
1	5058	157	99		143.724	5	26882	136	231		16.952
2	84728	157	825		93.595	6	245951	150	1815		8.377
3	312462	162	2343		74.498	7	148883	143	1155		8.095
4	298264	153	2343		49.450						

Figure 6. A polyacrylamide gel's protein bands' measurements for interaction of Moringa extract 10 g L^{-1} with potassium silicate 2 ml L^{-1}.



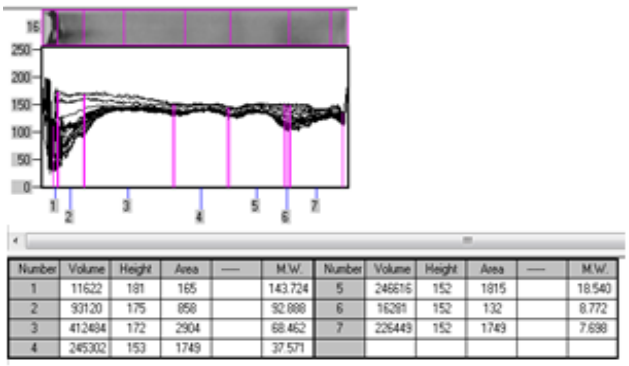
Number	Volume	Height	Area	----	M.W.	Number	Volume	Height	Area	----	M.W.
1	3260	126	66		143.724	5	11493	130	99		16.443
2	80040	146	792		93.595	6	280180	151	2145		9.252
3	380314	151	2871		66.948	7	115954	143	891		7.698
4	244806	140	1947		38.496						

Figure 7. A polyacrylamide gel's protein bands' measurements for interaction of Moringa extract 10 g L^{-1} with potassium silicate 3 ml L^{-1}.



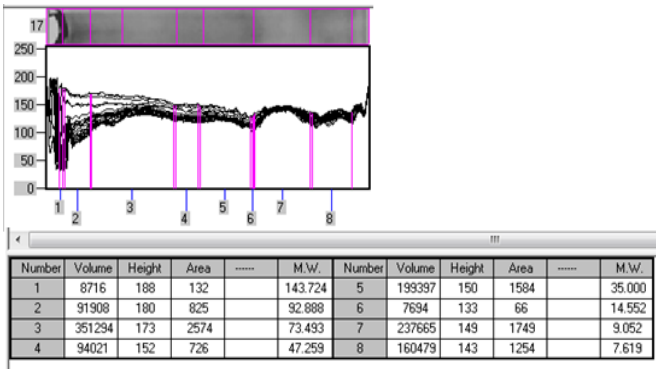
Number	Volume	Height	Area	----	M.W.	Number	Volume	Height	Area	----	M.W.
1	4175	185	66		143.724	5	271556	153	2244		32.166
2	78431	183	792		93.595	6	361066	152	2838		13.315
3	103826	173	825		76.824	7	28866	125	297		8.175
4	233743	168	1782		55.214						

Figure 8. A polyacrylamide gel's protein bands' measurements for interaction of Moringa extract 15 g L^{-1} with potassium silicate 1 ml L^{-1}.



Number	Volume	Height	Area	----	M.W.	Number	Volume	Height	Area	----	M.W.
1	11622	181	165		143.724	5	246616	152	1815		18.540
2	93120	175	858		92.888	6	16281	152	132		8.772
3	412484	172	2904		68.462	7	226443	152	1749		7.698
4	245302	153	1749		37.571						

Figure 9. A polyacrylamide gel's protein bands' measurements for interaction of Moringa extract 15 g L^{-1} with potassium silicate 2 ml L^{-1}.



Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	8716	188	132		143.724	5	199397	150	1584		35.000
2	91908	180	825		92.888	6	7694	133	66		14.552
3	351294	173	2574		73.493	7	237665	149	1749		9.052
4	94021	152	726		47.259	8	160479	143	1254		7.619

Figure 10. A polyacrylamide gel's protein bands' measurements for interaction of 15 g L-1 Moringa extract and 3 ml L-1 potassium silicate.

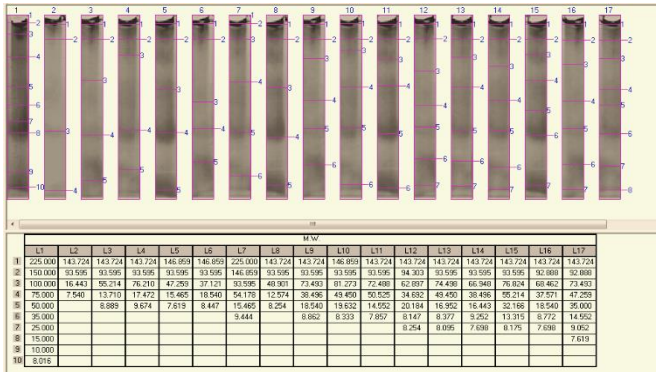


Figure 11. The number, positions, and molecular weights of protein bands in the leaves of banana seedlings treated with Moringa extract and potassium silicate (Photocapt programme).

***Conclusion*:** The application of Moringa extract and potassium silicate to banana seedlings leads to a quantifiable change in protein patterns, indicative of enhanced gene expression and protein synthesis. These changes are likely

responsible for observed improvements in plant growth and development, suggesting that such treatments could be valuable tools in sustainable agriculture practices for bananas. By harnessing the natural properties of Moringa extract and the beneficial effects of potassium silicate, farmers and researchers may improve crop yield, quality, and resilience in an environmentally friendly manner. This study underscores the potential of using plant extracts and mineral supplements as biostimulants in the agricultural sector, promoting not only the health and productivity of crops but also contributing to the broader goals of sustainable farming and food security.

***Author's contributions*:** Authors' contributions: Riyadh Mhawesh Al-Muhe and Mahmmod S. Abdul-Wahid: conceived and designed the experiments, performed the experiments, analyzed the data. and. wrote the paper, reviewed the manuscript. All authors read and approved the final manuscript.

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***Ethical statement*:** This article does not contain any studies with human participants or animal performed by any of the authors.

***Availability of data and material*:** We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere

***Code Availability*:** Not applicable

***Consent to participate*:** All authors are participating in this research study.

***Consent for publication*:** All authors are giving the consent

to publish this research article in JGIAS

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